

THE  
SCANDINAVIAN JOURNAL  
OF  
*Clinical & Laboratory  
Investigation*

EDITED BY  
THE SCANDINAVIAN SOCIETY  
FOR CLINICAL CHEMISTRY  
AND CLINICAL PHYSIOLOGY

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ROY HANSSON

Heparin-Induced Increase of  
Diamine Oxidase/Histaminase (EC 1. 4. 3. 6.)  
in Blood and Lymph

Scand. J. clin. Lab. Invest. - Vol. 31 - Suppl. 129 - 1973

SCLSAH 31 (129) 1-24 (1973)

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*Application to mail at second-class postage rates is pending at Boston, Mass.*

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ROY HANSSON

Heparin-Induced Increase of  
Diamine Oxidase/Histaminase (EC 1.4.3.6.)  
in Blood and Lymph



“For my own part, I am expecting that the new association which they have so convincingly established, between histamine and the heparin-containing mast-cells of the tissue, will be found to have an increasing importance, for assignment . . . of their various functional roles in a range of physiological and pathological reactions.”

*Sir Henry Dale*

Foreword to J. Riley. “The Mast Cells” (1959)

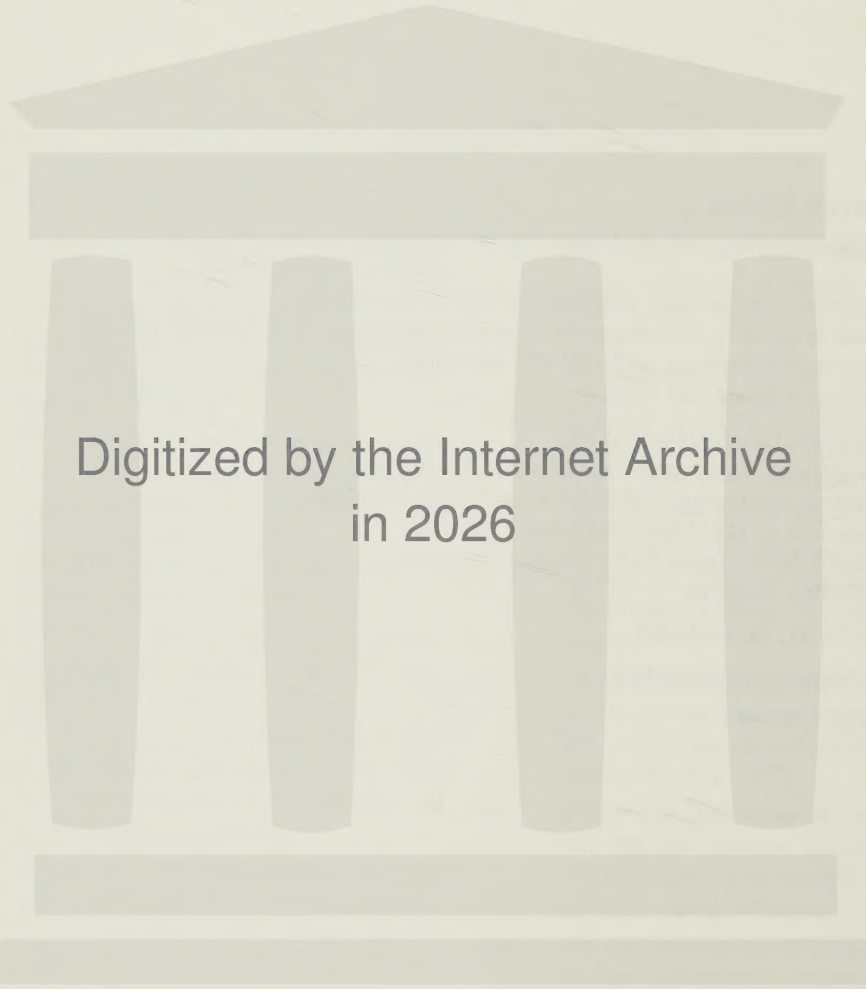
This survey is based on the following papers, which will be referred to in the text by their numerals:

- I. Heparin-induced diamine oxidase increase in human blood plasma. *Acta med. scand.* **180**, 533, 1966, in collaboration with C. G. Holmberg, G. Tibbling, N. Tryding, H. Westling and H. Wetterqvist.
- II. The effect of heparin on diamine oxidase and lipoprotein lipase in human lymph and blood plasma. *Scand. J. clin. Lab. Invest.* **21**, 17, 1968, in collaboration with O. Dahlbäck, G. Tibbling and N. Tryding.
- III. Diamine oxidase isoenzymes in human blood plasma. *Scand. J. clin. Lab. Invest.* **25**, 33, 1970.
- IV. Diamine oxidase in blister fluid. *Acta dermat.-venereol. (Stockh.)* **47**, 94, 1967, in collaboration with U. Kiistala, H. Rorsman and N. Tryding.
- V. Diamine oxidase (histaminase) in blister fluid and blood plasma during pregnancy. *Acta obstet. gynec. scand.* **48**, 19, 1969.
- VI. Diamine oxidase (histaminase) in human pregnancy. The activity of the enzyme in lymph and in blood plasma and the effect of heparin on the latter. *Acta obstet. gynec. scand.* **48**, 8, 1969, in collaboration with N. Tryding and Å. Törnqvist.
- VII. Diamine oxidase (histaminase) and heparin inhibition of gastric secretion in man. *Scand. J. clin. Lab. Invest.* **26**, 263, 1970, in collaboration with G. Sundström.
- VIII. Diamine oxidase in blood plasma in some vertebrates and *Anodonta cygnea* before and after injection of heparin. *Acta physiol. scand.* **74**, 533, 1968.
- IX. The effect of heparin and some related agents on diamine oxidase in rabbit blood plasma. *Acta physiol. scand.* **78**, 539, 1970.
- X. Heparin-induced increase of diamine oxidase in lymph and blood plasma of rat, guinea-pig and rabbit. *Acta physiol. scand.* **81**, 208, 1971.
- XI. Effect of organ removal or damage on the heparin-induced diamine oxidase (DAO) response in rat and rabbit. *Acta physiol. scand.*, in press.
- XII. On the elimination of transfused heparin-induced diamine oxidase (DAO) activity in rabbits. *Acta physiol. scand.*, in press.
- VIII—XII. In collaboration with H. Thysell.



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# Introduction

When Tryding in 1965 reported (64), that parenteral administration of heparin to man was followed by a transitory but rapid and strong increase of the diamine oxidase (= DAO, EC 1.4.3.6.) activity in blood plasma, these findings touched at least three research fields in medicine, viz anaphylactic shock, human pregnancy, and heparin-lipoprotein lipase interaction.

1. *The anaphylactic shock* reveals in its pathophysiology a number of characteristics, as studied in laboratory animals.

a) As early as about 1910 it was reported, that the canine blood — and lymph — was incoagulable during anaphylactic shock (9; 13). Thirty years later, this effect could be coupled with the presence of heparin in blood (35). In the blood of some other laboratory animals, during anaphylactic shock, metachromatic material, with heparin-like properties, could be isolated (46; 29; 25), although the presence of heparinemia during anaphylactic shock has been doubted, except in the dog (53). However, the degranulation of mast cells during anaphylactic shock and exteriorization of heparin from these into the tissue fluid seem to be generally accepted (52). Liberation of heparin, thus, is one of the pathophysiological events of the anaphylactic shock.

b) The histamine-inactivating enzyme (22), "histaminase" (7; 8) has — with some reservation (for ref. 38) — been considered identical with diamine oxidase (diamine:  $O_2$  oxidoreductase, deaminating, EC 1.4.3.6). Histaminase in

rabbit (55), in guinea-pig (43), and DAO in white rat (18) were demonstrated in the blood plasma of these laboratory animals during anaphylactic shock. In the guinea-pig the increased enzyme activity was demonstrated in blood plasma as soon as 15—20 seconds after the injection of the challenge dose of antigen. Logan remarked that "The prompt appearance of histaminase activity at a time of massive destruction of mast cells suggests that this activity may have been released by these cells. This possibility merits further study". In spite of this, the connection between the substances, liberated by the mast cells during anaphylaxis, and the increase of histaminase was not clarified by the time of Tryding's discovery of heparin-induced DAO increase in human blood plasma, after heparin injection. A research group, led by H. Giertz and F. Hahn, Freiburg i.Br., had however, shortly before, during studies on the anaphylactic shock in the guinea-pig, demonstrated that in this species only, heparin liberated histaminase from the liver (6).

Heparin, i. e. one of the substances liberated during the anaphylactic shock, was thus shown to bring about the mobilization of the DAO (histaminase) activity, i. e. another known participant in the shock, with a direct effect on histamine, the initially dominating principle. It was then considered important to know if the heparin-induced liberation of DAO was the rule, or an exception, within the vertebrate series in various (super-) classes (VIII). If a redistribution of DAO takes place after heparin injection, will its presence in the blood stream

then enhance the histamine catabolism in an organ relatively poor in DAO (VII)?

2. In *human* beings, increased DAO activity (pouvoir histaminolytique) has been observed in blood plasma during *pregnancy* (45). Determination of DAO in blood has, furthermore, been used as a routine analysis for the control of the feto-maternal unit (for ref. 60; 67; 69), or for the diagnosis and follow up to hydatiform mole (73; 63). In addition, an increased DAO activity in blood plasma has been reported, secondary to other extrauterine tumours (for ref. 2).

During pregnancy, large amounts of DAO are found in and around the decidua, membranes, and placenta (59). The DAO (histaminase) activity is conveyed by the uterine veins into the systemic circulation of the pregnant woman (62). After parturition, the elimination of the DAO (histaminase) activity has been followed by blood plasma analysis (1; 16). To what extent would intravenous injection of heparin influence the DAO levels of blood plasma; i. e. would the pregnancy-induced DAO depots add to the DAO response (VI)? How would the heparin-provoked DAO increase be eliminated, as compared to that of non-pregnant subjects and the spontaneous decline of DAO after parturition (I, III, VI)?

The presence of DAO activity in skin (61) and the contents of skin blisters (54) has been demonstrated. However, the DAO concentration is low enough to characterize the skin as poor in DAO activity. Would it be possible to influence the DAO activity in blister fluid, by means of intravenous injection of heparin (IV)? Or would it remain unchanged as an evidence that the molecular size of the enzyme precludes its dif-

fusion from the capillaries into the blister and as a sign that no local liberation of DAO occurs in the skin (IV)? Would the fluid of suction blisters in pregnant women covariate with the DAO activity in blood plasma (V)? Or would a bad penetrating power of the pregnancy-induced DAO molecule as well as its confinement to pregnancy-induced blood plasma debar the enzyme activity from blister fluid?

3. *Lipoprotein lipase (LPL) activity* has been demonstrated in blood plasma after the injection of heparin. The phenomenon was first described by P. Hahn in 1943 as an "Abolishment of alimentary lipemia following injection of heparin" (31). This post-heparin clearing factor has also been observed after liberation of endogenous heparin, *e. g.* during anaphylactic shock. In spite of this heparin-induced enzyme response, there seems to be no suggestions in the literature, before 1964, that analogous mechanisms might explain the DAO (histaminase) increase during anaphylactic shock (*vide supra*). Instead, the enzyme-releasing pharmacologic effect was to be demonstrated, unexpectedly, after intravenous injection of exogenous heparin for LPL (for ref. 41) as well as for DAO/histaminase (6; 64), leading to a new aspect on the mechanism of the anaphylactic shock. For that reason it was considered interesting to study some of the characteristics of the heparin-induced DAO increase in blood plasma, such as its time curve in some species (I, II, VII, IX) — in man including a comparison with the LPL curves as well (II) — its covariation with the activity of lymph (II, X), and, finally, its dependency of the dose of heparin and some related agents (I, IX).



# Present Investigations,

results and discussions, concerning  
DAO and its relations to heparin

## PHYLOGENETIC, MORPHOLOGIC, AND TOPOGRAPHIC ASPECTS

The diamine oxidase (DAO) activity of blood plasma was increased after intravenous heparin injection in all vertebrates under investigation, although there was a considerable species difference and some individual variation (I, VIII, IX, X).

Since the pioneer work of Zeller in 1938 (for ref. 74) it is known that the specific activity of DAO and the distribution of the enzyme among the (visceral) organs vary throughout the vertebrate series (for ref. 38). However, most data concerning organ content of DAO activity have been reported from investigations of few species and without consideration of extractability, of concomitant inhibitors or enzyme-destroying principles in the organs. The histological distribution within the cell is not known — obstacles to such studies have been reported (*e. g.* 56) — and the possible existence of stationary extracellular DAO activity in the organs is still unknown. Thus, there seems to be no valid morphological basis in the literature for a discussion of the sources of the heparin-induced DAO activity in vertebrate blood plasma.

In the guinea-pig Schmutzler & al. have demonstrated that the plasma DAO (histaminase) activity is liberated chiefly from the liver, after intravenous heparin injection (for ref. 56). In the rat (39; XI) and the rabbit (27; XI) evidence is given that extirpation of the intestine or occlusion of the visceral arteries will drastically reduce the plasma DAO activity. Furthermore it has been reported that the intestines of

rats and rabbits exhibit a distinctly lower content of DAO activity, after large doses of heparin, than is found in the control animals (39; 27). Neither in the rat nor in the rabbit did the liver seem to contribute materially to the plasma DAO activity after heparin injection (XI). These findings strongly indicate that as to the (visceral) sources of the increased blood plasma DAO activity after heparin injection, a considerable species variation exists.

Furthermore, we have not been able to obtain a purposeful correlation between the species-bound differences in the DAO response to heparin on the one hand and, on the other, literature data concerning phenomena such as sensitivity to histamine, tendency to anaphylactic reaction, the catabolic routes of histamine, sex, body temperature or reproduction.

The ubiquitous presence of mast cells throughout the vertebrate series has been accepted (52; 58; 23), although with some reservation (for ref. 36). The presence of heparin in mast cells is also well established (32; 72). In the guinea-pig, the role of endogenous heparin as a trigger substance for the release of DAO (histaminase) during anaphylactic shock has been demonstrated (25). Our findings, after exogenous heparin (VIII), indicate that analogous mechanisms are possible for other species as well. After all, increased plasma DAO (histaminase) levels have been reported during anaphylactic shock in the rat, the rabbit and the guinea-pig.

## LIBERATION AND TRANSPORT OF DAO

The relative volumes of distribution of heparin,



after iv injection, correspond to the extent of the plasma volume as shown in man, rabbit, and dog (21), and the blood plasma half lives of heparin amount to, very approximately, one hour. A rapid penetration of heparin into extravascular compartments is, consequently, less likely as an explanation of the earliest DAO increase in the blood of man and rabbit, although the data presented do not exclude a minor effusion of heparin. One possible explanation of the rapidly appearing DAO in post-heparin human and rabbit blood plasma (I, IX), might be mobilizable enzyme in the capillary walls of some organs. This would then be analogous to what has been assumed for lipoprotein lipase (LPL) (*e. g.* 24). A complete analogy between DAO and LPL release into blood does not exist in man, however, even if it has been reported that the "appearance curve of postheparin LPL activity can be divided into two main parts, the first a very rapid appearance rate between 0 and 6 min and thereafter a second slower appearance rate" (11). With similar doses of heparin we found that, whereas the activity of DAO in thoracic duct lymph was about one hundred times higher than in blood plasma, the LPL activity was definitely lower in lymph than in simultaneously collected blood plasma (II).

In the guinea-pig, the blood plasma DAO (histaminase) increase after heparin is far steeper than in *e. g.* man and rabbit (I, IX). Recently it was demonstrated, in guinea-pigs (28), that the esterase inhibitor  $\gamma$ -butyrolactone (butanoic acid, 4-hydroxy-, lactone) drastically reduces the heparin-induced release of DAO (histaminase). The metachromatic properties of postheparin blood plasma and the anticoagulatory characteristics were far less affected, if at all. These findings suggest another possibly mediating mechanism in the liberation of DAO (histaminase) in the guinea-pig.

Various theories have been advanced concerning the interaction of LPL and heparin, but no definite proof exists. The liberating mechanism of DAO, *in vivo*, after heparin injection, is even more unknown. The possibility of a DAO-heparin complex cannot be ruled out *in*

*vitro* (III), but such an interaction may be quite unspecific (5). The events *in vivo* await further elucidation, concerning the possible trigger enzymes, before a basis for discussion is warranted.

Whenever early sampling of blood has been performed after the heparin injection, in man and rabbit, we have found higher DAO activity in blood plasma than before the heparin. For example, in five rabbits given 0.8 IU heparin per g body-weight, the DAO activity in plasma was 6.5 times higher 0.5 minutes after the iv injection than before it;  $0.01 < p < 0.025$ , if a normal distribution of the difference is assumed. This finding is in contrast to a statement that, in the rabbit, the increase of histaminase in plasma does not occur until 10—15 minutes after the (iv) heparin injection (27). The discrepancy may depend on, *inter alia*, different sensitivities of Tryding's DAO and their histaminase methods, and possibly a lower pH optimum of the enzyme activity (with histamine as substrate) in blood samples taken at 15 minutes than in blood samples from 60 minutes after the heparin injection (27). As measured with our  $^{14}\text{C}$ -putrescine technique, however, some of the mobilizable DAO activity will apparently reach the blood stream at a time when the injected heparin has been in the blood compartment for only a very few circulatory cycles.

Gel filtration of postheparin and pregnancy DAO activities in human blood plasma did not indicate that the molecular size of the former enzyme variant is significantly different from that of the latter (III), and both types of DAO were shown to have a molecular size definitely larger than that of albumin. If the liberation of a macromolecule takes place from parenchymal cells or interstitial structures in the tissue, then the transport to the blood stream is expected to occur by way of the lymphatic vessels (*e. g.* 19). However, transcellular transfer or "fenestrated capillaries" or other kinds of lymphaticovenous communications may shunt part of the enzyme activity from the long and slow lymphatic channels. This fenestration of the capillaries has been reported to be several times more frequent on the venous than on the arterial end of the vessel in intestinal villi of mice and is

found in several organs where "there is likely to be interstitial accumulation of large molecules needing removal . . ." (17). Very little is, however, known concerning the transport capacity of this shunting.

In man, rabbit and rat such a release mechanism may exist with liberation of DAO from parenchymal cells, or interstitial structures, into the tissue fluid (II, X). Some of this liberated DAO activity may leak into the blood stream, whereas the highly concentrated remainder is conveyed by the lymph ducts. In the guinea-pig, however, the postheparin DAO activity is generally higher in blood plasma than in simultaneously collected lymph (X).

The rat and the rabbit, furthermore, differ from the guinea-pig both as to the organs which contribute to the postheparin DAO in plasma, and the shape of the heparin-induced DAO curve. Different access to the blood and lymph streams thus seems to exist for the liberated DAO in different species, possibly reflecting the "fenestration" of the contributing organ(s), or different sources in the same animal.

In the rabbit the possibility of different forms of blood plasma DAO (histaminase) after heparin has been demonstrated (27). Delayed penetration of heparin to the source of enzyme was also mentioned as a possible explanation of the second peak in this species.

The pregnant state in women does not seem to exert a major influence on the blood plasma DAO reaction, after heparin injection (VI). It is possible, however, that the maximal DAO response in plasma occurred somewhat earlier and its level was rather high. The flow of lymph, if accelerated, may be one possible reason for this. However, the eliminatory phase seemed to indicate the usual pattern with a virtually "monoexponential" run to the original pregnancy level. The data, thus, did not suggest a mixture of released DAO — of pregnant and of post-heparin DAO with different eliminatory rates (III) — to be eliminated during, or after, the observation period.

The DAO activity in the contents of blister fluid revealed a covariation with the heparin-induced (IV) as well as the pregnancy-depen-

dent enzyme activity (V) in blood plasma. No hints were given that, in the blister fluid, the heparin-induced variety would differ quantitatively in its ratio to the blood plasma enzyme, from pregnant DAO activity. As the distribution volume of the normally increased DAO activity during pregnancy seems to be the blood plasma compartment (VI), it is reasonable to assume the possibility that, in spite of their molecular size (III, 50), the blister plasma and that blister fluid may be regarded as an ultrafiltrate due to functional porosities in the filter.

Thus, as to the possible interactions between human pregnancy and the heparin-induced DAO reaction, there is at present very little evidence of such an interference.

## ELIMINATION OF DAO FROM THE BLOOD

Carlsten, Kahlson and Wicksell were the first to demonstrate that, in the dog, the activity of DAO (histaminase) in lymph is about one ten-fold higher than in the blood plasma of the animal (15). This was shown to apply to the species of the cat and the rabbit, as well (14). In man, data hitherto agree with an overspill of lymph DAO activity, at something like 0.7 U/l (II) conveyed by about 2 liters of lymph a day (10) into the blood stream where the average activity is almost 4 mU/l (68). It is, thus, possible to calculate the "clearance" of DAO to about 250 ml per min or, if the plasma volume is about 3.5 liters, a fractional rate of total plasma clearance at about 0.07 per min. These figures presuppose that the whole contribution of DAO occurs by lymph and is rather constant. If this was the case, the average infusion by the thoracic lymph of DAO into the blood stream would amount to less than 1 mU per min in man.

In some contrast to the described physiologic inflow, about 200—300 mU DAO activity were infused per minute to our human recipients during the transfusion of post-heparin plasma (III). The injected activity was, as it seems in man, eliminated in an exponential fashion with the mean "half life" in blood plasma at about

3 minutes. This would represent a fractional rate of plasma clearance of about 0.23 per min. The data did not indicate any inhomogeneity in the DAO activity of human plasma.

Rabbits, after transfusion of postheparin DAO, exhibited a two-component elimination (XII), the rapid phase of which had a half life of DAO at about 0.7 min, the slow one varying about 10—15 min (cf. 51).

It is, thus, an open question, if the physiological overspill of DAO activity represents the same form of molecules as that which is liberated by heparin. The transfused plasma contained not only DAO and heparin, but protamine in calculated equivalent amounts as well. However, in similar experiments with rabbit postheparin blood plasma transfusion, no clue was given that the addition of protamine would influence the rapid elimination of the DAO from the systemic circulation of the recipient (XII). The half-life of transfused DAO activity was about one minute in the rabbit.

The rate of elimination of postheparin DAO from blood and that of the physiological overspill-DAO seemingly differ. But it is by no means demonstrated that the overspill by the thoracic duct does dominate the physiological inflow of DAO into the systemic circulation. For that reason, the vaguely indicated clearance rate may be largely underestimated. Furthermore, no experiments with injection of e. g. thoracic duct lymph, containing hundreds of mU of "physiological" DAO, have been published. No conclusions can thus be drawn concerning different behaviour *in vivo* of physiological or overspill DAO and postheparin DAO.

The decline of the heparin-induced blood plasma DAO-activity curve, is determined by the eliminatory capacity, but the latter is counteracted by delayed DAO contribution, e. g. by the lymph, still tending to increase the enzyme activity in blood plasma 2—3 hours after the heparin injection (II). The "half-life" thus, is rather overestimated as judged from the curve.

The possibility exists that the presence of significant heparin concentrations in blood may retard the eliminatory process. Although no evidence concerning DAO in this respect seems

to have been published as yet, it has been suggested that heparin may retard the inactivation of plasma lipoprotein lipase during the passage through canine and murine livers (71; 47).

*In vitro*, addition of heparin, followed by protamine to blood plasma from a pregnant donor did not cause obviously different eliminatory rate after transfusion of the pregnancy-induced DAO (III). *In vivo*, administration of heparin to pregnant women was followed by a possibly accelerated DAO response in blood plasma, but the eliminatory rate was not altered in any obvious way; nor was the basal pregnancy-induced DAO level affected after the acute effect of the heparin injection had passed. It thus seems likely that heparin does not exert any significant influence upon the pregnancy-induced DAO.

Placental DAO (histaminase) in pregnant women is carried to the systemic circulation by the uterine veins (62). Of special interest in the present context is that — in a single observation — the thoracic duct DAO activity was lower than that of the blood plasma and about the same as in non-pregnant subjects (VI). It has also been demonstrated that, *post partum*, the decline of the DAO (histaminase) activity in blood plasma corresponds to the half life of about 27 hours (1; 16), whereas transfused pregnancy DAO disappears (in male subjects) with a half life of about 20 hours. The difference is small enough to be compatible with the possibility that the puerperal physiology (e. g. return of the plasma volume from the pregnant state, remaining DAO activity in the uterine wall, possibly contributing to the plasma DAO etc.) may lead to an underestimation of the plasma clearance, as deduced from the "half-life" values of the spontaneous return of DAO during the puerperal period (III).

It is known that the blood plasma DAO activity ( $= C U/l$ )\* is fairly constant during the last 15—20 weeks of pregnancy. If the plasma volume ( $V l$ ) remains sufficiently constant during the period of observation, this should mean

\* The activity at time zero  $= C_0$  and, at time  $t$ ,  $C_t U/l$ , respectively.



that a steady state exists for the turn-over of DAO activity. The quantity of DAO passing through the circulation ( $\dot{Q}$  U/unit of time) may be deduced from the following equation, provided that the elimination is a mono-exponential function, which is indicated by the experimental findings of other authors and us (*vide supra*);  $\lambda$ , time-unit<sup>-1</sup>, is the rate constant:

$$C_t \cdot V = C_0 V \cdot e^{-\lambda \cdot t} + \frac{\dot{Q}}{\lambda} (1 - e^{-\lambda \cdot t}),$$

and, if  $C$  remains constant,  $\dot{Q} = \lambda \cdot C \cdot V$  (for ref. *e. g.* 51). The mean blood plasma DAO level of normal pregnant women is, after the 20th week of pregnancy, about 1.4 U/l (and the individual data seem to vary according to the rules of a log-normal distribution; 20). The plasma volume can be experimentally determined with some precision, but the elimination constant is difficult to estimate during pregnancy. If for an average pregnancy woman, the plasma volume is assumed to be 3.5 l (33) during the period of investigation and if the similar results (half-life of DAO  $\sim$  1.1/day, corresponding to  $\lambda = 0.63$ ) concerning the half-life of transfused DAO activity into males or endogenous DAO in puerperal women, would be valid for the pregnant women, then in U/day  $\dot{Q} = 0.63 \cdot 1.4 \cdot 3.5 \sim 3$ . These hypothetical figures, however based on some valid data, would indicate that the daily contribution of placental DAO to the systemic circulation may be only a few times higher than is carried by the thoracic duct lymph.

Similarly, in man, for the plateau-like post-heparin plasma DAO activity (when inflow = elimination), if the half-life of DAO is 3 min in plasma, and finally, the maximal DAO activity is 2 U/l in a plasma space of 3.5 l, then  $\dot{Q} \sim 0.23 \cdot 2 \cdot 3.5 \sim 2$ , where  $\dot{Q}$  stands for U/min! That would mean a thousandfold increase of DAO inflow, as compared to the physiological overspill (1 day = 1440 min).

In DAO transfusion experiments on man, it was shown that the eliminatory rate of heparin-induced DAO activity was about 400 times higher than that of pregnancy DAO. It is interesting to note that during pregnancy, the

DAO activity in blood plasma increases from the mean value at almost 4 mU/l to an average of 1.4 U/l, i. e. nearly 400 times. During pregnancy the plasma volume gradually increases by about one third (33). As the concentration ratio of the two kinds of blood plasma DAO in question are inversely proportional to their extraction ratio, if a steady state is reached, this may be used as another argument for the possibility that rather similar amounts of spillover DAO might be brought from the placenta and from sources independent of pregnancy. It is also consistent with the possibility that the much higher levels of DAO activity in blood plasma during late pregnancy, as compared to the non-pregnant state, may depend more on different eliminatory rates of the two kinds of DAO enzyme than on the placenta as a cornucopia.

In the vertebrate series, the slope of the eliminatory phase of the post-heparin DAO curve seems to vary considerably. Determination of the disappearance of injected DAO activity (and heparin) might elucidate species differences, concerning height and duration of the DAO response, even as much as studies of the contribution of DAO have done.

Although some knowledge exists, concerning the eliminatory rate of DAO out of blood plasma, no information seems to be available to explain how, or where, the different forms of enzyme activity are eliminated. The molecular size makes it less likely that a significant quantity of the enzyme is excreted by the urine, and the reported findings of urinary DAO may depend on excretion of enzyme, produced by the kidney cells rather than eliminated from plasma. We have not been able to demonstrate significant amounts in human urine of DAO even in heparin-stimulated normal subjects. Inhibitors are found in urine and are eliminated after dialysis (66). However, in a case of chyluria, after heparin injection, large amounts of DAO were recovered (data to be published), without need of preliminary gel filtration or other precautions for the elimination of inhibitors.

Rabbit bile, after heparin injection, contains low DAO activity but no inhibitors, which could be demonstrated by the addition of bile

to samples of known DAO activity. In the rabbit urine no DAO activity was found, and thus no excretory routes explaining the entire elimination of postheparin DAO from the blood plasma have, as yet, been demonstrated.

Our clearance studies, however, indicate that a large minute flow of blood plasma through the enzyme-eliminating organ(s) is necessary to match the fractional rate of total plasma clearance of post-heparin DAO.

The problem why the basal plasma DAO activity is reported as slightly elevated in some cases of cirrhosis of the liver, is still unexplained in man (42). The high postheparin DAO values in rat blood plasma, after hepatectomy, may depend on an impaired mechanism of elimination as well (XI). For both of these conditions an increased contribution by the lymph might be another explanation of the high DAO level.

Further studies seem to require gentle purification and labelling of different kinds of DAO, before a solution may be found to the question of how and where DAO is eliminated.

#### FURTHER KINETIC ASPECTS AND A MECHANICAL MODEL

Evidence has been presented that the major heparin-induced DAO activity comes from visceral organs such as the liver in the guinea-pig (*e. g.* 56), and the intestines in the rat (39; XI) and the rabbit (27; IX).

In man, rabbit, rat, and guinea-pig (the only species hitherto investigated in this respect) the DAO activity increase is found both in lymph and in blood (II, X). In the guinea-pig, however, the DAO increase in lymph is smaller than that in plasma, whereas the DAO level in the other species is ten to hundred times higher in lymph than in blood plasma. Liberation of DAO, thus, seems to occur both into interstitial fluid — to be transported and concentrated during the formation of central lymph — and more directly into the blood stream, as is evident during the early ascent of the plasma DAO activity.

In man, rat and rabbit, the individual post-heparin DAO curve in blood plasma generally describes a two-humped course. The second maximum coincides with the time of maximal

inflow of thoracic duct lymph, the withdrawal of which from the systemic circulation is followed by a poor response in these species (II, X). Experimental data thus suggest that the second maximum is dependent on the retarded lymph-transported DAO amounts. It cannot be denied, however, that the possibility of a slow heparin access to the DAO depots (27) may be valid as an explanation, just a slow liberation of DAO both into the blood stream and tissue fluid might be another.

Several problems are still unsolved. Nothing is known of the duration of the heparin stimulus on the DAO-releasing mechanism or the length of the release process, regeneration of enzyme, etc. The efficiency of the wash-out of the released DAO activity by blood and lymph; the permeability of the membranes between tissue fluid and blood plasma near the hitherto unidentified histological structures that store the

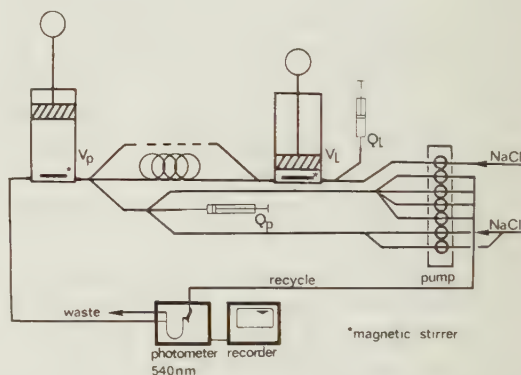


Fig. 1. Two compartments,  $V_p$  and  $V_L$ , were made of modified large syringes supplied with in- and outflow openings and a magnetic stirrer.  $V_L$  (about 3 ml) was perfused with a small volume of saline solution (*e. g.* 1.2 ml/min).  $V_p$  (about 70 ml) was perfused with a larger volume of saline (7.8 ml/min) and a recycling solution from  $V_p$  (15.6 ml/min) and a coil-delayed solution from  $V_L$  (1.2 ml/min). At time zero a large quantity ( $Q_L$ ) of  $HbO_2$  was rapidly injected into  $V_L$  and a small one ( $Q_p$ ) into  $V_p$ . The optical density of the outflow from  $V_p$  was recorded (Beckman DB with flow cell and 10" lin/log recorder). The fluid was forced through the system by a proportioning pump (Technicon) with standard tubing and connections. The time lag from  $V_L$  to the light path was  $\sim 20$ s.

mobilizable enzyme; all of these factors add to the difficulties of a discussion of the DAO kinetics.

If, however, the net effect of the mentioned structural and physiologic uncertain factors are of minor importance, it might be possible to visualize the release and elimination of DAO activity in a mechanical model (Fig. 1) under the following assumptions.

1. DAO is released from structures into blood plasma ( $Q_p$ ), and into the tissue fluids ( $Q_L$ ) (It is not known whether  $Q_p$  comes from the vessel walls or passes through them; furthermore it is not known if  $Q_p + Q_L$  is the whole amount of primarily released DAO activity.)
2.  $Q_p$  is eliminated from the blood plasma at one fractional rate ( $\lambda$ ), and so is  $Q_L$  after gradual access to this compartment (However, the influence of heparin on the eliminatory rate of DAO from plasma is unknown.)
3. The transport of  $Q_L$  may occur by the main lymphatic trunks (delay is indicated by mixing coil), or be short-cut *via* lymphaticovenous shunts (interrupted line).
4. The flow of tissue fluid in the resting subject may be regarded as a rather constant transport effect through a space the volume of which remains practically unchanged during the period of observation.

The tubing and volumes in the mechanical model were chosen, in this example, so as to mimic, within practical limits, the conditions of the post-

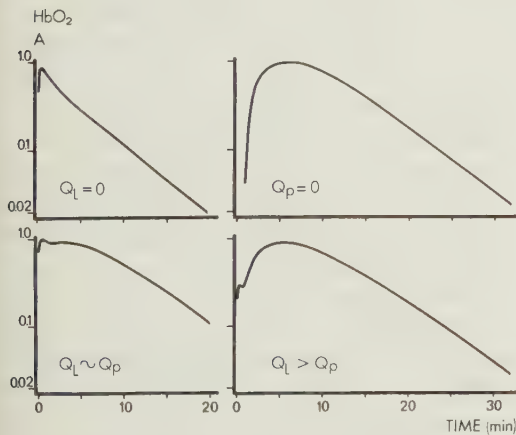


Fig. 2. The log optical density of  $HbO_2$  vs time, for varying proportions of  $Q_p$  to  $Q_L$  (see Fig. 1).

In the lower quadrants  $\frac{Q_L}{Q_p}$  was 1.4 (left) and 6, respectively.

heparin DAO response in human blood plasma. About one third of the systemic volume, if the dead space of tubing is considered, was substituted with clean saline, while roughly the same volume was wasted.  $Q_p$  and  $Q_L$  were varied (Fig. 2) to give satisfactory maximum values of optical density; the duration of the injection was 1 to 2 s.

In Fig. 2 the logarithm of optical density is chosen for ordinate in order to put the system on a par with the log. DAO activity in most of our reports. It is evident that even when  $Q_L \sim Q_p$ , or somewhat larger, the undulation indicating a second maximum (due to inflow from  $Q_L$ ) may be easily overlooked, if the observations are infrequent or the analytical methods are less exacting.

When a contribution of oxyhemoglobin to  $V_p$  comes from  $V_L$ , a delay occurs, depicted by the coil and, possibly, counteracted by shunts. If mixing and transport were ideal, and the volume of the transport tubing negligible, the outflow from  $V_L$

$$\text{would have the concentration } \frac{Q_L}{V_L} \cdot e^{-\lambda \cdot t}$$

where  $\lambda$  is the fractional rate of clearance of  $V_L$ . Due to the transport defects, the gradient of hemoglobin concentration is blurred. The entry of fractions of  $Q_L$  is, thus, smoothed.

If it is permissible to compare the mechanical model with the blood plasma DAO responses to heparin injection in the vertebrate series (VIII), the guinea-pig and possibly the cow (and goat?) exhibit a response similar to the optical density when  $Q_L = 0$ , or  $Q_L \sim Q_p$ . In the fowl, however, the individual curves undulate with a second maximum indicated, after a dip, following the abrupt rise initially. The same two-phased run of the curve is seen in some of the rat, cat and dog experiments as well. If this comparison is suggestive of a second, late contribution of DAO activity, it must be kept in mind, that other explanations may be applicable as well.

In the right half of Fig. 2 the theoretical possibility of  $Q_p \sim 0$  is considered (top) as well as

the possibility  $Q_p < Q_L$  (leading to  $\frac{Q_p}{V_p} \ll \frac{Q_L}{V_L}$ ).

No reports seem to be available, as yet, indicating a heparin-induced release of DAO activity exclusively into the tissue fluids with only a late contribution due to slow transport mechanisms or a delayed heparin-effect on remote



DAO stores (27). On the other hand, the possibility of  $Q_p < Q_L$  (i. e.  $\frac{Q_p}{V_p} \ll \frac{Q_L}{V_L}$ ) seems to be

a reality in some species. The lower curves in Fig. 2 seem to be prevalent among the species, hitherto investigated; the example to the right may represent man and rabbit.

In our mechanical model  $Q_p$  stands for the quantity of DAO of prompt release into the plasma compartment and  $Q_L$  for the late contribution. The duration of the oxyhemoglobin injection was, in our model, very short in comparison to the eliminatory rates of the distribution volumes  $V_p$  and  $V_L$ , in order to simplify the present discussion. The principles of a more elaborate device are actually built in, permitting a protracted injection of  $Q_p$  and  $Q_L$  according to what will be known concerning the release from the DAO-rich structures. A protracted release of  $HbO_2$ /DAO would be followed by less sharp gradients in the present mechanical model.

To interpret or explain the *in vivo* release of DAO activity after heparin injection, further details must be known. It is not known when the delivery of enzyme from the DAO-rich structures began, when it ceased, nor the quantitative variation during the liberation process. A complete correlation between the model system and the *in vivo* events during heparin-induced liberation of DAO activity is thus not warranted as yet.

The eliminatory rate of injected species-specific DAO has been studied only in man and in rabbit. Very little is known, as yet, concerning its dependence upon the load of transfused DAO activity, or heparin, or both, in blood plasma. In the guinea-pig, however, the influence of injected pea-seedling DAO upon the anaphylactic shock has been studied, as well as its elimination from plasma of recipient dogs — where the half life was something like 40 min (70). The DAO response, after heparin, is not directly proportional to the dose, or log dose (e. g. IX) but more complex, within limits increasing with increasing doses. These findings may be consistent with a delaying effect of heparin on the elimination of DAO activity from blood plasma. It may help to explain another finding, as well, if the explanation is not a con-

tinued release of regenerated DAO or engagement of other stores. In the guinea-pig we found that the height of the plasma DAO peak after 0.2 U heparin per g was not much lower than the one after 1.6 U per g body weight (VIII). According to repeated communications (e. g. 56) 0.5 U heparin per g will exhaust almost the entire store of histaminase in the liver, and our finding thus far, agreed with the reports. However, the rate of return to normal differed. The guinea-pig given the low heparin dose returned to normal DAO levels with an enzyme "half-life" of about one half hour, whereas the animals, given the large heparin dose, remained at maximal levels for more than one hour. These findings may warrant further investigations on the dose-response problem of plasma DAO activity after heparin. This is especially so since the guinea-pig, after lymph deprivation (X), gave no clues to the possibilities of a retarded inflow by DAO-rich lymph, indicating other sources of the enzyme. Questions of that kind at present seem to require further biological elucidation before a mathematical model may be devised in order to define the kinetics of the DAO release, after heparin injection.

## CONCLUDING REMARKS

Intravenous injection of heparin into a vertebrate is followed by a transient increase of the DAO activity in blood plasma in all individuals hitherto examined. These include single samples from bone fishes, amphibia and birds, and several kinds of mammals. Irrespective of its biological significance, the reaction at present seems to be inherent throughout the vertebrate series.

Other authors have shown that after heparin injection the DAO (histaminase) is passed into the blood stream from the liver in the guinea-pig (56), but from the intestines in the rat (39) and in the rabbit (27). Our own observations (X, XI) have confirmed the importance to the post-heparin DAO levels in blood of some visceral organs in the rat and rabbit.

The transport of DAO (histaminase) by lymph into the blood stream has been known for more than twenty years (15; 14). The postheparin increase of DAO activity in visceral or thoracic

duct lymph may be significant, in spite of the small lymph flow, for the height of the DAO levels in blood plasma (II, X).

The effect of injected heparin (and some other polyanions) in this respect may consequently be regarded as a re-distribution of the DAO activity from its original position(s) to a circulating state (*ad utrumque paratus*). The increase in blood plasma enzyme activity is somewhat dramatic. Within a few minutes the DAO content of blood plasma increases several times. This high level is maintained likewise in a dose-dependent fashion, during quarters to multiples of hours, in spite of a rather short average life of the mobilized DAO in plasma — a biological parallel to the penal servitude of the Damaids!

The biological importance of DAO is not settled yet, in spite of various theories, ever since its discovery (74; 36; 38; 12). From a modest position as an indiscriminate scavenger enzyme of polyamines, diamines and histamine, the interest in the first-mentioned substances as important metabolic regulators has given new import to the enzyme. Its presence in the placenta has, together with important findings concerning the histamine metabolism (for ref. 36), stimulated the curiosity concerning the biological importance of DAO. In the absence of proof of its necessity, it is still more difficult to evaluate the metabolic effect of the post-heparin DAO mobilization, except from the aspect of detoxification in connection with increased plasma levels of histamine, *e. g.* shock due to anaphylaxis, anaphylatoxin or histamine (26).

If a substance is cleared from the blood by an organ, the clearance depends on the perfusion, as well as the efficiency of the extraction during the blood passage. If only a small fraction of the cardiac output flows through the organ and is diminished by shock, the efficiency of the clearing process will suffer, unless the extraction compensates. A mobilization of DAO from the organs might, hypothetically, increase the effectiveness of detoxication if the time of availability of the substrate to the enzyme is increased to the full circulatory cycle, and if the concentration of enzyme in relation to the

substrate decreased, and, finally, the pH in blood plasma were within optimal conditions. Communications proving the effect of heparin-induced DAO-mobilization on the total histamine metabolism in various species, seem to be lacking. However, valuable results have been presented, concerning the "clinical" signs and the plasma histamine level in the guinea-pig during shock due to anaphylaxis, anaphylatoxin and histamine (26). The effect of iv injected doses of heparin on the histamine levels in plasma and on the clinical picture was significant for the anaphylatoxin and histamine shock, but only in one of the two series of anaphylactic guinea-pigs a reduction of the plasma histamine content was noted.

One of these authors later reported that "diamine oxidase (histaminase) plays a distinct but limited role in plasma histamine clearance in anaphylaxis in guinea-pigs as well as after histamine injection" and, "under the influence of heparin, the rate of oxidative deamination in the body is probably doubled by histaminase release into plasma such as occurs in anaphylaxis" (30).

Our findings in man, that the basal — but not the histamine-stimulated — gastric acid secretion is diminished after heparin injection, unless aminoguanidine is administered as well, would best fit with the assumption that mobilized DAO may exert a limited, but statistically significant effect on remote organs normally poor in measurable amounts of the enzyme (VII). These findings have recently received experimental support by other authors (40). It is thus possible that the circulating post-heparin DAO may exert a protective effect, within limits, on some histamine sensitive organs or structures.

Endogenous heparin is found in the mast cells. However, simultaneous evaluation of tissue heparin and the number of mast cells in small tissue samples, have demonstrated considerable variation and the possibility of heparin being present outside the mast cells in dog and rat (34). In spite of, or in the light of, these data, two classical statements may be revised. "There is no easy entry for heparin into the blood in most species. The function of heparin must lie in the tissues" (52) The presence of

heparinemia in normal vertebrates has been a matter of dispute, and even concerning the heparinemia during the anaphylactic shock in laboratory animals there seems to be some controversy (57; 46; 29; 25) except for the dog.

During anaphylactic shock, increased DAO has been demonstrated in the blood plasma of rabbit, rat, and guinea-pig (*op. cit.*), and in man a DAO (histaminase) elevation in blood has been reported (12; 12a; this has been found also by us in post mortem blood from a young man suffering from Brazil-nut allergy with a fatal outcome in anaphylactic shock). In the guinea-pig, the presence in blood of heparin, as well as its mediating role, in DAO (histaminase) release during anaphylactic shock, has been proven by the Freiburg research team (57; 25; 26; 30). Similar evidence for other species seems to be lacking in the literature.

The classical event with interaction between histamine, heparin and DAO is thus described as a whole, or in its facets, after massive destruction of the mast cells, "the emergency kit" (58). During this event a highly active vasodilating amine with target structures in smooth muscle is liberated to exert a local effect and gain a rather early entry into the systemic circulation. The heparin, even in the dog, appears later in the blood (52).

In seeming contrast to delayed contribution of heparin to the blood stands the very rapid appearance there of DAO (histaminase) by 20 seconds after the challenge dose in anaphylaxis (43). If heparin, one mediator of the DAO liberation, really acts in the tissue, then the passage of a larger enzyme molecule would indicate a far less "easy entry into the blood". In some

species the contribution of DAO by the lymph has been demonstrated, after iv heparin injection (II, X), and during anaphylactic shock (48).

During anaphylactic shock in the dog, heparin, but no significant amount of DAO (histaminase), has been demonstrated in blood plasma (48; 56). This somewhat paradoxical finding may depend on a number of factors, such as sensitivity to heparin, amounts of mobilisable DAO, eliminatory rates, etc., and has not as yet been clarified.

The last decade has seen ingenious analytical methods for determination of histamine (43; 4) and diamine oxidase (49; 65; 68; 3), leading to better sensitivity and precision, often in spite of less work load per analysis. It is the hope of the author, that the following subjects, which have been partially elucidated in the present communication, might be better explained in the near future:

- a) The mechanism of liberation of DAO activity from its stores, the histology of which is yet unknown.
- b) The transport routes to plasma, and from it, in a quantitatively satisfactory approach.
- c) The question of DAO elimination from the blood stream as a function of the DAO and heparin concentrations.
- d) The biological significance of the DAO response to heparin, endogenous as well as exogenous, in other laboratory animals than guinea-pigs.
- e) The biological significance of heparin therapy in patients with an allergic diathesis.



## Summary of Findings and Conclusions (in Papers I-XII):

Intravenous injection of heparin (or related substances, IX) is followed by an increased blood plasma DAO activity which

- 1) is the rule in the vertebrate series, i. e. in all (super-) classes of vertebrates examined by us (I; VIII);
- 2) is dose-dependent (I; VII; VIII; IX);
- 3) is dependent on the vertebrate species under investigation, both as to the rapidity of the DAO response, its height and duration; however, within each species a considerable individual variation is seen (I; VII; VIII; IX);
- 4) in man seems to be rather uninfluenced by the presence of the DAO-rich placenta, and adjacent structures, during pregnancy (VI);
- 5) in man seems to be correlated to the DAO in the fluid of spontaneous and suction-induced skin blisters (IV), similarly to the correlation between pregnancy-provoked DAO in plasma and that of suction blisters (V);
- 6) is dependent on the contribution of thoracic duct lymph, in the rabbit and probably in man and the rat, as well, i. e. the species in which a much higher postheparin DAO activity is seen in lymph than in blood plasma; the guinea-pig seems to be independent of a DAO contribution by central lymph due to its lower DAO activity, as compared to guinea-pig blood plasma (II; X);
- 7) is mainly of intestinal origin in the rat and the rabbit (XI), which confirms reports by other authors;
- 8) in man is concomitant with a reduction of the basal, (but not of the submaximally histamine-induced) gastric acid production; the heparin-induced inhibition was, however, abolished by the DAO-inhibitor aminoguanidine (VII);
- 9) is eliminated several hundred times more quickly, in man, than pregnancy-provoked bloodplasma DAO, in spite of a rather similar molecular size (III);
- 10) is different from pregnancy-provoked blood plasma DAO as to electrophoretic migratory properties in agarose gel but not as to major reaction kinetics (III);
- 11) is, when transfused into man (III) or rabbit (XII), eliminated many times faster from the blood of the recipient subject as compared to the spontaneous return to basal level after heparin stimulation of the individual (III);
- 12) in man describes a pattern different from that of lipoprotein lipase (LPL) activity in blood plasma, possibly depending upon, *inter alia*, different routes of enzyme contribution (II);
- 13) may be a reaction, simulating the endogenously elicited and possibly purposeful DAO liberation into the blood during the events of the anaphylactic shock, with its simultaneous liberation of histamine and heparin from their origin, the mast cells.

## Acknowledgements

Docent Nils Tryding awakened my interest in the field of work upon which the present studies are based. Since then, his never failing interest, encouragement and helpful criticism have formed a deeply appreciated support.

Professor Håkan Westling has stimulated the progress of the work, at times spurring, at others providing laboratory facilities and advice, experimental as well as stylistic.

Professor Carl Gottfrid Holmberg, my teacher and former chief, has generously put his scientific experience as well as the resources of his laboratory at my disposal during the initial phase of these studies.

Professor Nils Alwall enabled the experiments on animals in work VIII—XII by hospitable sharing of grants and operating rooms.

Professor Per Arne Öckerman, Docent Kjell Jacobsson, Docent Olav Thulesius, Docent Erik Olof Arvidsson and Dr Dick Heinegård in co-operation with Professor Sven Gardell have, in many various ways, willingly helped me as referees.

Mrs and Dr Louis Milner, M.R.C.S.L.R.C.P. D.A.D.P.H., have revised the language of the manuscript.

To all these friends I wish to express my sincere gratitude, as well as to the helpful and cooperative team at the department of clinical chemistry and many colleagues at Centrallasarettet, Växjö.

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